charles river

BIOLOGICS TESTING SOLUTIONS

Program Overview: Immune Checkpoint Antibodies

Project Package

- Tumor models for drug discovery
- Physicochemistry/structure
- Function – biological activity assays
- Formulation
- Preclinical *in vivo* package
- Preclinical/clinical laboratory support package
- Lot release and stability testing

Charles River has a range of services to support our clients' anti-immune checkpoint monoclonal antibody development programs, from tumor mouse models through *in vivo* preclinical studies and support assays for both preclinical and clinical sample analysis to GMP facilities for performing characterization and lot release testing. By combining the experience found across the company, we have developed a comprehensive program that clients can use to support the development of these molecules.

Overview

Examples of monoclonal antibody therapeutics interacting with T cell signaling receptors include Ipilimumab (αCTLA4, or cytotoxic T-lymphocyte 4), which broadens the melanoma-reactive CD8+ T cell response, as well as Nivolumab and Pembrolizumab (αPD-1, or programmed cell death protein 1). PD-1 inhibitors activate the immune system to attack tumors by blocking the PD1/PD1L binding mechanism. Both molecules have an inhibitory effect in the cancer immunity cycle. Examples of immune checkpoint activators targeted by agonist mAbs include αGITR and αOX40. Glucocorticoid-induced TNFR-related protein (GITR) shows an increased expression upon T cell activation and inhibits the suppressive activity of regulatory T cells and thereby extends the survival of effector T cells. OX40 is a member of the TNFR superfamily with increased expression of this receptor upon T cell activation. OX40 also inhibits the suppressive activity of regulatory T cells. Multiple other molecules modulating the T cell response are currently in development by many research groups. Although the targets of these molecules are different, the goal is the same: modulate T cell reactivity to tumors by blocking inhibitory pathways or enhancing stimulatory pathways.

Supporting Our Clients' Programs

As a global company with extensive scientific expertise, we guide clients from discovery to approval and provide continuity for their entire program. This means that they can work with just one provider instead of investing time and money qualifying multiple vendors.

Navigating the regulatory landscape is also key to being first to market. Our scientific advisory services group is able to efficiently support clients through the many guidelines aimed specifically at the development of immune checkpoint antibodies.

Development Package

Monoclonal antibody therapeutics targeting immune checkpoint inhibitors or activators are a heterogeneous class of T cell modulating drugs. Therefore, the individual programs required for development and characterization as well as for biosimilarity assessment and lot release need to be tailored to the individual molecules. The program outlined below is based on the class of drugs, scientific experience, and published guidance documents.

Table 1: Development and Characterization Package for Anti-Immune Checkpoint Inhibitors

Tumor Models for Drug Discovery	
Mouse models for efficacy, PK, safety, MTD, MOA	
More than 20 characterized syngeneic models	
Humanized knock-in models where the mouse gene is replaced by a human gene (e.g., CTLA-4)	
Humanized mice: human immune cells engrafted into immunodeficient mice	
Physicochemistry/Structure	**Function – Biological Activity Assays**
• Protein quantity and purity • Molecular weight determination by MS • Amino acid sequence by LC-MS/MS and Edman degradation • Glycosylation and other PTMs analysis • Physicochemical properties • Aggregation analysis by SEC-MALS and AUC	• Pathway-specific reporter assays • Antibody-dependent cell-mediated cytotoxicity (ADCC) assay • Complement-dependent cytotoxicity (CDC) assay • Antibody-dependent cellular phagocytosis (ADCP) assay • Apoptosis assay • Flow cytometry binding assay • Cell sorting • Fc receptor assays
Preclinical *In Vivo* Package	**Preclinical/Clinical Laboratory Support Package**
• Pharmacology/pharmacodynamic PK/PD study • Four-week comparative toxicity study • Tissue cross-reactivity	• Bioanalysis and pharmacokinetic (PK) analysis • Immunogenicity • Pharmacodynamic (PD) (e.g., flow cytometry, *ex vivo* recall response, antigen challenge models, cytokine analysis) • Receptor occupancy
Formulation	**Lot Release and Stability Testing**
• Preformulation • Stress studies • Product formulation (or reformulation)	• Pilot studies • Release/stability methods development and validation • Routine testing • Stability testing including accelerated stress condition testing with mode of action assays